REMARKS

No new matter is added by the amendments to the claims. Claims 1-10, 12-14, 17-24 and 26-35 are currently pending in this application. Originals of Figures 1 and 2 filed with the 1.132 Declaration filed April 2, 2001 are enclosed.

Claim Objections

Claims 11 and 15 have been rewritten as new claims 30 and 31, respectively, incorporating all previous amendments. The misspelled words in claims 19 and 28 have been corrected or deleted. Claim 22 has been amended as suggested by the Examiner.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 1-14 and 16-25 remain rejected under 35 U.S.C. § 112, second paragraph, as indefinite.

Claims 1, 11, 17-19 and 26 were rejected for reciting "cytoplasmic tail". Applicants respectfully traverse this rejection. The claims have been amended to define the cytoplasmic tail by function, as a localization signal to target or localize the chimeric enzyme, and hence the first glycosyltransferase, to a selected cell compartment or organelle so that this first enzyme is topographically positioned to compete with the "different enzyme" for a common substrate to achieve the result defined, namely the reduction of the major epitope responsible for hyperacute rejection.

Applicant submits that the term "cytoplasmic tail" would be clear to a person skilled in the art, having regard to the field in which the present invention resides, carbohydrate biology and transplantation of organs. It would be apparent to a skilled artisan that glycosyltransferases are a class of proteins, i.e. type II transmembrane protein having the generic structure already shown in applicant's reply to the office action dated March 9, 2000 (see applicant's response dated 10 July 2000 at page 11, a further copy of which is provided for convenience). For the Examiner's reference, applicant submits copies of articles discussing typical type II transmembrane protein structure, including the cytoplasmic tail. See especially the abstracts and introductions in the enclosed references by D. Aoki *et al*, Proc. Natl. Acad. Sci. USA, 89:4319-4323, 1992; N. Osman *et al*, J. Biol. Chem., 271(51):33105-33109, 1996; A.S. Opa *et al*, J.

Biological Chemistry, 275: 11836-11845, 2000; L. M. Gastinel *et al* EMBO Journal, 20(4): 638-641, 2001.

In brief, these references describe transmembrane type II proteins as sharing a common structural domain organization consisting of an N-terminal cytoplasmic tail, a single transmembrane region connected by a stem or stalk region to a C-terminal catalytic domain, as shown in the diagram in applicant's response of 10 July 2000. See also page 103 from chapter 4 in "The Leucocyte Antigen Facts Book", 1997 (enclosed).

Thus, a person skilled in the art would understand that the cytoplasmic tail of the glycosyltransferases contemplated by the present invention would be confined to the N-terminal sequence of the glycosyltransferase in question. Such information may also be obtained from databases publicly available e.g. the web site of the Department of Biochemistry at The University of Western Ontario. Copies of excerpts from this site are enclosed for the Examiner's reference.

Accordingly, applicant submits that the term "cytoplasmic tail" is clear. As indicated above, a person skilled in the art would understand the part of the different glycosyltransferase (a type II protein) which corresponds to the cytoplasmic tail, and which may be used in accordance with the present invention.

Claims 8, 9, 11, 18, 19, 23, 24, 26, 28 and 29 were rejected as indefinite for various reasons. Applicant has amended the claims as suggested by the Examiner. Applicant thanks the Examiner for his guidance in progressing the claims. Concerning claim 9 (and dependent claims), Applicant has amended it such that the $\alpha(1,3)$ galactosyltransferase is defined as the enzyme that catalyses the production of a gal$\alpha(1,3)$gal epitope that causes hyperacute rejection.

Claim 11 has also been amended by deleting "t" from Ht; H transferase is defined in the new claim. The claim has also been amended to clarify the relationship between the N-terminal of GT and the localization signal of the chimeric enzyme, and to clarify the relationship between the transmembrane, stem and catalytic domains of H transferase and the catalytic domain of the enzyme.

Concerning claim 26 (see page 6, first full paragraph of the Office Action), applicant submits that it is intended that only the first glycosyltransferase may optionally be a carbohydrate modifying enzyme. It is operably linked to the cytoplasmic tail of the different glycosyltransferase. The cytoplasmic tail then localizes the first glycosyltransferase, or the

carbohydrate modifying enzyme at the same cellular position as the "native" different glycosyltransferase such that the first glycosyltransferase, or the carbohydrate modifying enzyme will compete with the native, different glycosyltransferase for substrate. In the instance of claim 26, the carbohydrate modifying enzyme will compete with the different glycosyltransferase to produce e.g. a sulphated oligosaccharide in place of a gal α(1,3) gal epitope on a cell that thus have the potential to escape hyperacute rejection.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-15, 17-24 and 26-29 are rejected for introducing new matter. The Examiner states that there does not appear to be support for methods or compositions comprising limitations to "cytoplasmic tail", "carbohydrate modifying enzymes" and "specific for a trans Golgi". Applicants respectfully traverse this rejection.

Applicant submits that the use of a cytoplasmic tail from a type II glycosyltransferase is described throughout the present specification. For example, see page 5 lines 34-35 of the PCT publication, WO 98/05768, where it is stated that localization signals generally comprise amino terminal tails of a glycosyltransferase. As already explained above, glycosyltransferases are type II membrane proteins the structure of which is well understood by a skilled artisan. In particular, it would be understood by such a person that the cytoplasmic tail and the amino terminal sequence of such a membrane protein are synonymous. See for instance, chapter 4 in "The Leucocyte Antigen Facts Book" (*supra)*, particularly Fig 1 and page 103. Thus, applicant submits that there is support for "cytoplasmic tail".

The Examiner's attention is further directed to Example 1 beginning at page 13, where it is clearly shown that chimeric enzymes comprising cytoplasmic tails of glycosyltransferases such as "GT", "HT" were generated. See page 16 lines 6-11 and Fig. 1. See also page 18 lines 8-12 where it is stated that the cytoplasmic tails dictate the pattern of carbohydrate expression. Further, see page 19 lines 22-24 where it is stated that the order of glycosylation is altered by exchanging the cytoplasmic tails.

The Examiner also alleges that there is no support for "carbohydrate modifying enzymes". Applicant respectfully disagrees. Page 5 lines 22-24 describe a glycosyltransferase as a polypeptide having the ability to "move carbohydrates from one molecule to another". It would be understood that the polypeptide (being a glycosyltransferase) inevitably and by its

nature, acts on an oligosaccharide or carbohydrate substrate (see enclosed page 559 of text book reference: "Biochemistry" by CK Matthews and KE van Holde, The Benjamin/Cummings Publishing Company, Inc., CA 94065, USA, 1990). By its action, a sugar molecule that is initially linked to a nucleotide is "moved" to a non-reducing oligosaccharide. Thus, the carbohydrate molecule (the oligosaccharide) to which a sugar has been moved is modified by the action of the glycosyltransferase, which can be called a carbohydrate modifying enzyme.

Applicant further submits that the term "carbohydrate modifying enzymes" is inherent in the description of the invention, as a skilled artisan would readily understand that glycosyltransferases, including sialyltransferases, galactosyl sulphating enzymes or phosphorylating enzymes, especially when read in the context of the present invention, modify carbohydrates such as glycoproteins, proteoglycans and glycolipids. These enzymes modify these different classes of carbohydrates by adding sialic acid derivatives, sulphate groups or phosphate groups to the sugar residues in these carbohydrate classes respectively. Such carbohydrate modifying enzymes may be used to generate desired glycosylation patterns as disclosed at page 12 lines 14-16 for instance. See also underlined text in the attached copies of pages 90, 91 and 96 of "Molecular Glycobiology" by Fukuda and Hindsgaul, previously submitted with applicant's response of 30 March 2001.

In response the Examiner's allegation that there is no support for localization of the chimeric enzyme of the invention to a Golgi compartment, applicant refers to page 19 lines 28 to 30. Applicant further submits that it is known to a person skilled in the art that the glycosyltransferases contemplated by the invention translocate to the trans section of the Golgi compartment. The Examiner is further referred to page 8 of applicant's submission dated 30 March 2001, which was filed in response to the final office action, mailed 30 January 2001. There, the translocation of glycans as they are assembled, beginning from the ER to completion in the Golgi, and the role of glycosyltransferases were summarised, and served to show that the glycosyltransferases contemplated by the invention act on substrates located in the TGN. See also the relevant pages from Fukuda, *supra.*

Claims 1-14 and 17-25 remain rejected under 35 U.S.C. § 112, first paragraph, as not enabled. The Examiner maintains that the specification does not reasonably provide enablement of the broad scope of recited chimeric enzymes comprising any and all glycosyltransferase

functional domains other than those comprising localization domains of α(1,3) galactosyltransferase and/or catalytic domains from fucosyltransferases. Applicants respectfully traverse this rejection.

As already stated above, the glycosyltransferases are transmembrane (more specifically Golgi) enzymes. This is also clearly shown in A.S. Opa *et al, supra*. The Examiner's attention is directed to lines 8-9 of the abstract and the first paragraph of the main text of this reference for instance. See also the first sentence at second paragraph of the full text, where it is stated that all Golgi glycosyltransferases cloned to date are type II membrane proteins containing a common structure in terms of the various domains defined by the protein sequence of each enzyme. Thus, a person skilled in the art would know how the various domains of such enzymes can be interchanged in accordance with the present invention to achieve the result desired, i.e. a reduction in the epitope responsible for hyperacute rejection.

As an illustration, a skilled artisan would know that a Type II protein typically consists of the amino acid residue, Met, at the NH_2 terminus. This is immediately followed by a sequence of charged amino acid residues that represent the cytoplasmic region, the last residue being positively charged. What comes after the last of this sequence of residues is a transmembrane sequence of 16-22 hydrophobic amino acids. The last hydrophobic residue is followed by a positively charged residue. Hence the hydrophobic, transmembrane sequence is bordered at each end by positively charged residues. The transmembrane region is followed by the catalytic domain. Having this knowledge, a skilled artisan would be able to identify the cytoplasmic region and the catalytic domain of a Type II protein, and by following the teachings in the present specification, construct a nucleic acid or produce a chimeric molecule as presently claimed.

In regard to the Examiner's comments at page 10 of the office action on "carbohydrate modifying enzymes", applicant submits that a skilled artisan, having a knowledge of the protein sequence and structure of such enzymes would be able to operably link the catalytic domain of, for example, a phosphorylating enzyme to a localization signal of a glycosyltransferase as contemplated by the present invention, thereby generating a chimeric enzyme that will reduce the gal epitope by phosphorylating the sugar acceptor that normally accepts the terminal gal sugar following catalysis by α(1,3)-glycosyltransferase.

In conclusion, applicant submits that the claims are enabled. Having regard to the detailed knowledge of glycosyltransferases, i.e. type II membrane proteins and information thereon that are publicly available, a skilled artisan would be able to practise the invention. See, for example, the first full paragraph at column 2 of the reference by AS Opa *et al.* The first sentence states that all type II membrane proteins known to date contain "a short-terminal cytoplasmic domain, ...carboxyl-terminal catalytic domain...". In addition, several references on the structure of such proteins are also given. A person skilled in the art would be able, from such information, to determine the appropriate portions or domains from each glycosyltransferase that could be operably linked to form chimeric enzymes in accordance with the present invention.

Although applicants maintain that the claims were enabled as written, to further prosecution, the claims have been amended to recite that the product is a galα(1,3) gal epitope reactive with antibodies that cause hyperacute rejection. Applicants respectfully request withdrawal of this rejection.

CONCLUSION

In view of the amendments and remarks made herein, it is respectfully submitted that the application is in condition for allowance. Notification to that effect is earnestly requested.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

Please cancel claims 11 and 15, amend claims 1, 8, 9, 17, 18, 19, 22, 23, 24, 26, 28 and 29, and add new claims 30-35 as follows:

1. (TWICE AMENDED) A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a first glycosyltransferase and a localization signal of a different glycosyltransferase, [said localization signal comprising a cytoplasmic tail of said different glycosyltransferase, whereby said nucleic acid is expressed in a cell wherein said chimeric enzyme is located in a] wherein expression of said nucleic acid in a cell results in localization of said chimeric enzyme by said localization signal in the same cell compartment or organelle [where it is able to compete] in which the different glycosyltransferase is naturally present so as to allow competition for substrate [with the different glycosyltransferase and wherein the different glycosyltransferase is located in the same compartment or organelle as said chimeric enzyme,] between the first glycosyltransferase and the different glycosyltransferase [resulting] so as to result in reduced levels of a product from [said] the different glycosyltransferase when compared with the level of said product in a cell wherein the chimeric enzyme is not expressed, wherein said product [is an] comprises a galα(1,3) gal epitope reactive with an antibody that causes hyperacute rejection,

the improvement characterized in that the localization signal comprises a cytoplasmic tail of the different glycosyltransferase to localize the first glycosyltransferase at said cell compartment or organelle for said competition .

8. (TWICE AMENDED) The nuclei acid according to claim 1, wherein the localization signal is from [a] the same cell [that belongs to the same species] type as the cell of claim 1.

9. (TWICE AMENDED) The nucleic acid according to claim 1, comprising a sequence encoding the catalytic domain of H transferase and a nucleic acid sequence encoding a localization signal from α(1,3)-galactosyltransferase [,which transferase] that catalyses the

production of [an] a galα(1,3) gal epitope reactive with an antibody [to thereby] that causes hyperacute rejection.

17. (TWICE AMENDED) A method of producing the nucleic acid according to claim 1, comprising the step of operably linking a nucleic acid sequence encoding a catalytic domain from a first glycosyltransferase to a nucleic acid sequence encoding a localization signal of a different glycosyltransferase, said localization signal comprising a cytoplasmic tail of said different glycosyltransferase to localize the first glycosyltransferase at the same cell compartment or organelle in which the different glycosyltransferase is naturally present.

18. (TWICE AMENDED) A method of reducing an amount of a carbohydrate exhibited on a surface of a cell, said method comprising causing a nucleic acid to be expressed in said cell wherein said nucleic encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localization signal of a different glycosyltransferase, [said localization signal comprising a cytoplasmic tail of said different glycosyltransferase, whereby said chimeric enzyme is located in a] wherein expression of said nucleic acid in said cell results in localization of said chimeric enzyme by said localization signal in the same cell compartment or organelle [where it is able to directly compete] in which the different glycosyltransferase is naturally present so as to allow competition for substrate [with said different glycosyltransferase, and wherein said different glycosyltransferase is located in the same compartment or organelle as said chimeric enzyme,] between the first glycosyltransferase and the different glycosyltransferase [resulting] so as to result in reduced levels of [a product] said carbohydrate from [said] the different glycosyltransferase when compared with the level of said [product] carbohydrate in a cell wherein the chimeric enzyme is not expressed, wherein said [product is] carbohydrate comprises [an] a galα(1,3) gal epitope reactive with an antibody that causes hyperacute rejection,

the improvement characterized in that the localization signal comprises a cytoplasmic tail of the different glycosyltransferase to localize the first glycosyltransferase at said cell compartment or organelle for said competition.

19. (TWICE AMENDED) A method of producing a cell from a donor species which is immunologically acceptable to a recipient species by reducing levels of carbohydrate on said cell, wherein said carbohydrate is capable of stimulating recognition of the cell as non-self by the recipient, said method comprising causing a nucleic acid to be expressed in said cell wherein said nucleic encodes a chimeric enzyme which comprises a catalytic domain of a first glycosyltransferase and a localization signal of a different glycosyltransferase, [said localization signal comprising a cytoplasmuc tail of said different glycosyltransferase, whereby said chimeric enzyme is located in a] wherein expression of said nucleic acid in said cell results in localization of said chimeric enzyme by said localization signal in the same cell compartment or organelle [where it is able to directly compete] in which the different glycosyltransferase is naturally present so as to allow competition for substrate [with said different glycosyltransferase, and wherein said different glycosyltransferase is located in the same compartment or organelle as said chimeric enzyme,] between the first glycosyltransferase and the different glycosyltransferase [resulting] so as to result in reduced levels of [a product] said carbohydrate from [said] the different glycosyltransferase when compared with the level of said [product] carbohydrate in a cell wherein the chimeric enzyme is not expressed, wherein said [product is] carbohydrate comprises [an] a galα(1,3) gal epitope reactive with an antibody that causes hyperacute rejection,

the improvement characterized in that the localization signal comprises a cytoplasmic tail of the different glycosyltransferase to localize the first glycosyltransferase at said cell compartment or organelle for said competition.

22. (AMENDED) [A non-human transgenic animal, organ or] Isolated cells comprising the nucleic acid according to claim 1.

23. (TWICE AMENDED) An expression unit that expresses a nucleic acid according to claim 1 [, which when used to transform] in a cell [results] resulting in [a cell which is imunologically acceptable to an animal having] reduced levels of a carbohydrate on its surface, which carbohydrate [is recognized as non-self by said species] comprises a galα(1,3) gal epitope reactive with an antibody that causes hyperacute rejection.

24. (TWICE AMENDED) A [retroviral-packaging cassette,] retroviral construct or retroviral producer cell comprising the expression unit according to claim 23.

26. (AMENDED) A nucleic acid encoding a chimeric enzyme, wherein said enzyme comprises

a catalytic domain of a first glycosyltransferase or a carbohydrate modifying enzyme, and

a localization signal of a different glycosyltransferase, said localization signal comprising a cytoplasmic tail of said different glycosyltransferase,

[whereby said nucleic acid is expressed in a cell wherein said chimeric enzyme is located in a] wherein expression of said nucleic acid in a cell results in localization of said chimeric enzyme by said localization signal in the same cell compartment or organelle [where it is able to compete] in which the different glycosyltransferase is naturally present so as to allow competition for substrate [with the different glycosyltransferase and wherein the different glycosyltransferase is located in the same compartment or organelle as said chimeric enzyme,] between the first glycosyltransferase or the carbohydrate modifying enzyme and the different glycosyltransferase [resulting] so as to result in reduced levels of a product from [said] the different glycosyltransferase when compared with the level of said product in a cell wherein the chimeric enzyme is not expressed, wherein said product [is] comprises [an] a gal α(1,3) gal epitope reactive with an antibody that causes hyperacute rejection,

the improvement characterized in that the localization signal comprises a cytoplasmic tail of the different glycosyltransferase to localize the first glycosyltransferase or the carbohydrate modifying enzyme at said cell compartment or organelle for said competition.

28. (AMENDED) A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a [fucosyltansferase] fucosyltransferase and a localization signal from α(1,3) galactosyltransferase [whereby] wherein expression of said nucleic acid [is expressed] in a cell [wherein] results in localization of said chimeric enzyme [is located in a] in the same cell compartment or organelle [where it is able to compete for substrate with the] in which α(1,3) galactosyltransferase [and wherein the α(1,3) galactosyltransferase is located in the

same compartment or organelle as said chimeric enzyme] is naturally present so as to allow competition for substrate between the fucosyltransferase and the α(1,3) galactosyltransferase [resulting] so as to result in reduced levels of a product from the α(1,3) galactosyltransferase when compared with the level of said product in a cell wherein the chimeric enzyme is not expressed, wherein said product [is] comprises [an] a galα(1,3) gal epitope reactive with an antibody that causes hyperacute rejection.

29. (AMENDED) A nucleic acid encoding a chimeric enzyme, wherein said chimeric enzyme comprises a catalytic domain of a first glycosyltransferase and a localization signal of a different glycosyltransferase, [said localization signal being specific for a trans Golgi and comprising a cytoplasmic tail of said different glycosyltransferase, whereby said nucleic acid is expressed] wherein expression of said nucleic acid in a cell [wherein] results in localization of said chimeric enzyme by the localization signal [is located] in [the] a trans Golgi cell compartment in which said different glycosyltransferase is naturally present so as to allow competition [where it is able to compete] for substrate [with the different glycosyltransferase and wherein] between the first glycosyltransferase and the different glycosyltransferase [is located in the same compartment or organelle as said chimeric enzyme, resulting] so as to result in reduced levels of a product from said different glycosyltransferase when compared with the level of said product in a cell wherein the chimeric enzyme is not expressed, wherein said product [is] comprises [an] a galα(1,3) gal epitope reactive with an antibody that causes hyperacute rejection,

the improvement characterized in that the localization signal comprises a cytoplasmic tail of the different glycosyltransferase to localize the first glycosyltransferase at said trans Golgi cell compartment for said competition.

30. (NEW) The nucleic acid according to claim 1, wherein the localization signal encoded by the nucleic acid is the NH_2 terminal cytoplasmic tail of GT and the catalytic domain encoded by the nucleic acid is the transmembrane, stem and catalytic domains of H transferase.

31. (NEW) A nucleic acid according to claim 1, wherein the localization signal is selected from the group consisting of MNVKGR (SEQ. ID. No. 11), MNVKGK (SEQ. ID. No. 12) and MVVKGK (SEQ. ID. No. 13).

32. (NEW) The nucleic acid according to claim 1, wherein the first and the different glycosyltransferase are each selected from a galactosyltransferase or a fucosyltransferase.

33. (NEW) The method according to claim 18, wherein the first and the different glycosyltransferase are each selected from a galactosyltransferase or a fucosyltransferase.

34. (NEW) The method according to claim 19, wherein the first and the different glycosyltransferase are each selected from a galactosyltransferase or a fucosyltransferase.

35. (NEW) The nucleic acid according to claim 29, wherein the first and the different glycosyltransferase are each selected from a galactosyltransferase or a fucosyltransferase.